In one aspect, the invention encompasses a peptide library comprising a collection of structurally constrained peptides. Each peptide member of the library comprises amino acid sequence C1-A1-A2-$(A3)_n$-A4-A5-C2 (SEQ ID NO:1), wherein

N.E

A1, A2, A3, A4, and A5 are naturally occurring L-amino acids;

the amino terminus of Cysteine C1 is optionally protected with an amino protecting group;

the carboxy terminus of Cysteine C2 is optionally protected with a carboxy protecting group;

A1 and A5 are selected from the group consisting of amino acids W,Y, F, H, I , V and T;

Please replace the paragraph beginning at page 12, line 26, with the following rewritten paragraph:

E2

A 16-mer peptide derived from the protein ubiquitin but with a statistically more common turn sequence (MQIGVKNPDGTITLEV (SEQ ID NO: 41)) did form a highly populated hairpin in water (ca. 80%). but the hairpin did not have the same strand register as in the native protein (Searle *et al.* (1995) *Nat. Struct. Biol.* 2:999-1006). Another group studied a similar peptide in which the turn region was replaced with several sequences (MQIGVKSXXKTITLKV (SEQ ID NO: 42)), wherein XX = pro-ala or pro-gly; Haque & Gellman (1997) *J. Am. Chem. Soc.* 119:2303-2304). Evidence for the hairpin structure, with native strand register, was observed for turns containing D-amino acids but not for L-amino acid sequences. No population estimates were given in this study.

Please replace the paragraph beginning at page 12, line 33, with the following rewritten paragraph:

E3

Several groups have studied model peptides based originally on a sequence from the protein tendamistat. The peptide YQNPDGSQA (SEQ ID NO: 26) shows NMR evidence of a small population of hairpin in water (Blanco *et al.* (1993) *J. Am. Chem. Soc.* 115:5887-5888; de

Alba *et al.* (1995) *Eur. J. Biochem* 233:283-292; Constantine *et al.* (1995) *J. Am. Chem. Soc.* 117:10841-10854; Friedrichs *et al. J. Am. Chem. Soc.* (1995) v 117, pp. 10855-10864). A variant of this peptide with strand residues of higher expected β-propensity (IYSNPDGTWT (SEQ ID NO: 27)) was compared to a second peptide with a different turn sequence (IYSNSDGTWT (SEQ ID NO: 28)). Both peptides were estimated by NMR as 30% hairpin in water (de Alba *et al.* (1996) *Fold. Des.* 1:133-144). Further variation of this peptide, predominantly in the turn sequence, yielded hairpins of various structures and mixed populations. Generally no one conformer population exceeded 50% (de Alba *et al.* (1997) *J. Am. Chem. Soc.* 119:175-183). In a final study, the three N-terminal residues in peptide ITSNSDGTWT (SEQ ID NO: 29)) were replaced with various sequences. Again, mixed conformers were frequently observed and populations of a given hairpin conformer were generally less than 50%: one peptide (YITNSDGTWT (SEQ ID NO: 30)) did form a register-shifted hairpin that was highly populated (80%; de Alba *et al.* (1997) *Protein Sci.* 6:2548-2560). The authors of these studies conclude that conformational preferences of the turn residues dominate cross-strand interactions in determining the stability of hairpins, at least in these short model peptides.

Please replace the paragraph beginning at page 13, line 11, with the following rewritten paragraph:

Analysis of hairpin sequences in crystal structures has allowed the design of a different series of β-hairpin peptides. The target structure was a type I' turn flanked by three-residue strands. Arg-gly sequences were added to the ends to improve stability. The peptide RGITVNGKTYGR (SEQ ID NO: 31) is partially folded into a hairpin conformation (about 30%) as determined by NMR (Ramirez-Alvarado *et al.* (1996) *Nat. Struct. Biol.* 3:604-612). The importance of strand residues is indicated by replacement of the ile and val, the lys and tyr, or all four residues with alanine. None of the alanine-substituted peptides showed any tendency to form a hairpin. The same authors reported a second series of experiments in which position i+1 of the turn was varied (asn to asp, ala, gly or ser). No peptide was more structured than the original sequence with asn in the turn (Ramirez-Alvarado *et al.* (1997) *J. Mol. Biol.* 273:898-912). A review describing this work stated that adding glu-lys pairs to the termini of the model

peptide stabilized the hairpin but did not give further details (Ramirez-Alvarado *et al.* (1999) Bioorg. Med. Chem. 7:93-103).

Please replace the paragraph beginning at page 13, line 23, with the following rewritten paragraph:

Another model peptide series (RYVEVXGOrnKILQ (SEQ ID NO: 32)) has yielded evidence for hairpin formation in water. Residue X as D-pro or L-asn yields characteristic NOEs and alpha-H shifts, but the L-pro peptide is unfolded. No population estimates are given, but D-pro appears to give the more stable hairpin (Stanger & Gellman (1998) *J. Am. Chem. Soc.* 120:4236-4237).

Please replace the paragraph beginning at page 13, line 26, with the following rewritten paragraph:

A designed 16-residue peptide (KKYTVSINGKKITVSI (SEQ ID NO: 33)) based on the met repressor DNA binding region formed a hairpin structure in water with an estimated population at 50% at 303 K. Truncation of one strand showed that the turn was populated without the strand interactions, although to a lesser degree (35%). An analysis of the thermodynamic parameters for hairpin formation showed that folding is enthalpically unfavored and entropically driven, with $\Delta G = 0.08$ kcal/mol at 298 K (Maynard & Searle (1997) *Chem. Commun.* 1297-1298; Griffiths-Jones *et al.* (1998) *Chem. Commun.* 789-790; Maynard *et al.* (1998) *J. Am. Chem. Soc.* 120:1996-2007).

Please replace the paragraph beginning at page 13, line 33, with the following rewritten paragraph:

A final hairpin peptide (GEWTYDDATKTFTVTE (SEQ ID NO: 34)) derived from the B1 domain of protein G (GB1) has some features relevant to the peptides of the invention. Unlike the above described model hairpins, the GB1 hairpin has four threonine residues at hydrogen-bonded sites in the strands, including one thr-thr cross-strand pair. This is generally believed to be an unfavorable pairing. In addition, there are trp-val and tyr-phe pairs at adjacent nonhydrogen-bonded sites that might interact to form a small hydrophobic core. The reported data indicate that the GB1 peptide formed a well-populated hairpin (about 50%) in water. The

data are consistent with native strand pairing (Blanco *et al.* (1994) *Nat. Struct. Biol.* 1:584-590). A denaturation study of the GB1 peptide allowed estimation of 80% hairpin at 273 K, and analysis of the data (assuming $\Delta Cp = 0$) yielded $\Delta H = -11.6$ kcal/mol, $\Delta S = -39$ cal/mol K: i.e., folding is enthalpically driven and entropically disfavored (Munoz *et al. Nature* (1998) v 390, pp. 196-199). The relative roles of enthalpy and entropy are reversed compared to the met repressor peptide described above.

Please replace the paragraph beginning at page 14, line 20, with the following rewritten paragraph:

The structure of a hexapeptide (Boc-CL-Aib-AVC-NMe) was determined crystallographically, revealing a type II' turn and β-sheet geometry (Karle *et al. J. Am. Chem. Soc.* (1988) v 110, pp 1958-1963). An octapeptide with the same cysteine spacing (ACSPGHCE (SEQ ID NO: 35)) was studied by NMR, and has a similar structure with a turn centered on pro-gly (Walse *et al.* (1996) *J. Comput.-Aided Mol. Des.* 10:11-22). Peptides of the form Ac-CXPGXC-NHMe (SEQ ID NO: 43) were evaluated by measurement of disulfide exchange equilibria, which indicated turn preferences between peptides of as much as 1 kcal/mol (Milburn *et al.* (1987) *J. Am. Chem. Soc.* 109:4486-4496).

Please replace the paragraph beginning at page 14, line 27, with the following rewritten paragraph:

An eleven-residue cyclic peptide (CGVSRQGKPYC (SEQ ID NO: 36)) based on the gene 5 protein from M13 is stably structured in aqueous solution, as demonstrated by NMR analysis. The cyclic peptide adopts a structure that is quite similar to the corresponding protein loop. The authors claim that well-defined β-hairpin structure had not been previously reported for any unprotected disulfide-constructed cycle (Rietman *et al.* (1996) *Eur. J. Biochem* 238:706-713). This peptide has a val-pro pair at the nonhydrogen bonded sites nearest to the cysteines.

Please replace the paragraph beginning at page 14, line 32, with the following rewritten paragraph:

Cyclization of peptides corresponding to loops from Linulus anti-lipopolysaccharide factor (LALF) based on X-ray structure yielded potent lipid A binders. There is no evidence for

structure in these peptides. Several of the peptides have aromatic-aromatic pairs at the nonhydrogen-bonded sites nearest the cysteines; however, the most potent (GCKPTFRRLKWKYKCG (SEQ ID NO:37)) has a pro-tyr pair (Ried *et al.* (1996) *J. Am. Chem. Soc.* 271:28120-28127).

E10 Cont

Please replace the paragraph beginning at page 15, line 1, with the following rewritten paragraph:

E11

Disulfide-cyclized peptides from the hairpin region of a rabbit defensin have antibacterial activity exceeding (about 5 to 10-fold) that of the linear analogs. Circular dichroism spectroscopy indicates some non-random structure in phosphate buffer. The more potent peptide (CAGFMRIRGRIHPLCMRR (SEQ ID NO: 38)) has a gly-pro pair at the nonhydrogen bonded sites nearest to the cysteines (Thennarasu & Nagaraj (1999) *Biochem. Biophys. Res. Commun.* 254:281-283).

Please replace the paragraph beginning at page 15, line 6, with the following rewritten paragraph:

E12

A final study describes several peptides from the loops of domain 1 of human CD4. In addition to a disulfide constraint, the authors have added exocyclic aromatic amino acids to the peptide termini. For example, a peptide covering CD4 residues 39-44 was constrained as FCNQGSFLCY (SEQ ID NO: 39). No evidence for structure is given, but one cyclic peptide (FCYICEVEDQCY (SEQ ID NO: 40)) was reported to antagonize both normal CD4 interactions and those involved in CD4-mediated cell entry by HIV (Zhang *et al.* (1996) *Nature Biotechnology* 14:472-475; Zhang *et al.* (1997) *Nature Biotechnology* 15:150-154).

Please replace the paragraph beginning at page 35, line 4, with the following rewritten paragraph:

E13

Libraries of random peptides fused to the gene 8 protein of the filamentous bacteriophage M13 were produced by Kunkel mutagenesis of plasmid pS1302b, a derivative of pS349 (U.S. patent application No. 09/380,448 which claims priority to U.S. Patent Application Nos. 60/103,514 filed October 8, 1998 and 60/134,870 filed May 19, 1999, incorporated herein by

reference). Plasmid pS1302b includes the tac promoter and malE leader sequence of pS349. The hGH sequence and Gly/Ser-rich linker sequence of pS349 were replaced by the sequence:

5'-TAA-TAA-TAA-ATG-GCT-GAT-CCG-AAC-CGT-TTC-CGC-GGT-AAA-GAT-CTG-GGT-GGC-GGT-ACT-CCA-AAC-GAC-CCG-CCA-ACC-ACT-CCA-CCA-ACT-GAT-AGC-CCA-GGC-GGT-3' (SEQ ID NO: 24)

Please replace the paragraph beginning at page 35, line 17, with the following rewritten paragraph:

The form of random peptides was therefore $XCTWX_4LTCX$. A library of 10^9 to 10^{10} individual transformants was prepared by previously described methods (U.S. patent application No. 09/380,447). Approximately one-third of individual clones encoded a functional peptide sequence. The remainder were starting template, contained stop codons, or contained single nucleotide deletions. The library size is thus adequate to include several copies of each possible random sequence.

In the Claims

Please cancel claims 4, and 15-18 without prejudice or disclaimer.

Please amend claim 1 as shown below.

1. (Amended) An isolated library of structurally-constrained cyclic peptides, wherein each said cyclic peptide comprises an amino acid sequence C1-A1-A2-$(A3)_n$-A4-A5-C2 [SEQ ID NO:1], wherein

C1 and C2 are cysteines;

A1, A2, A3, A4, and A5 are naturally occurring L-amino acids;

A1 and A5 are independently amino acids W, Y, F, H, I, V, or T;

A2 and A4 are independently amino acids W, Y, F, L, M, I, or V;